Volume 12

Washington University
Undergraduate Research Digest

Spring 2017

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Intracellular Localization of *Staphylococcus aureus* in Osteoclasts

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Osteomyelitis (OM) is inflammatory infection of the bone caused by *Staphylococcus aureus* (*S. aureus*) which can lead to prolonged hospitalization, sepsis, and even death. Historically, *S. aureus* was thought to be an extracellular pathogen, yet new research has shown that *S. aureus* is internalized into osteoclasts, the cells that destroy bone, and avoid cell defense mechanisms to remain alive intracellularly. The purpose of this study was to investigate how *S. aureus* evades the cellular defense system by studying where *S. aureus* resides within osteoclasts. The location of the bacteria within the cell, particularly the acidification status of the vesicle in which the bacteria are confined, is thought to determine the ability of the cell to combat bacterial growth. To determine the location of *S. aureus* within osteoclasts, I used fluorescence-based confocal microscopic imaging of the fluorescent dye Lysotracker, which marks lysosomes, in osteoclasts. Specifically, I infected primary murine osteoclasts and their precursors with the GFP-expressing MRSA strain USA300 to assess the degree of co-location of bacteria with acidified vesicles. Osteoclasts were fixed at 1.5 hours, 3 hours, 6 hours, and 18 hours post-infection for image analysis of the localization of intracellular *S. aureus* in lysosomes over time. I found that *S. aureus* is localized to lysosomes in osteoclasts at 1.5 hours and 3 hours of infection, but at 18 hours of infection is not localized to lysosomes. This indicates that *S. aureus* may avoid the cellular defense system by avoiding progression of endocytic vesicles to lysosomes or escaping the endocytic vesicle system in osteoclasts. To further assess the endocytic vesicular location of *S. aureus* in osteoclasts late in infection, I will next use antibodies for markers of earlier endocytic vesicles.